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Viability of the *Lactobacillus rhamnosus* IL1 Strain in Simulated Gastrointestinal Conditions

E. Vamanu and A. Vamanu

University of Agronomic Sciences and Veterinary Medicine,
Faculty of Biotechnology, Bd. Mărăşti No. 59, District 1, Bucharest, Romania

Abstract: The aim of this study was to determine viability in the transit of the *Lactobacillus rhamnosus* IL1 strain through the stomach and the small intestine. The preservation of the viability of the *Lactobacillus rhamnosus* IL1 strain in gastrointestinal conditions is one of the main characteristics of the strain, in order to obtain probiotic products. The tests were performed with a cell suspension kept in NaCl 0.5%. The pepsin influence was determined at different pH values, as well as the pancreatin influence in the presence of bile salts. The influence of casein and mucin was also established. The results were read using the ColonyQuant and they were registered in the log (CFU mL^{-1}). Greater viability was preserved in case of mucin which was confirmed by the calculation of the mathematical parameters of viability and mortality, according to whether mucin is used or not. The main conclusion is that the tested strain maintains its viability, even at pH ranging between 1.8-2 and at even greater concentration of bile salts, of 2-3 mg mL^{-1}. These results are confirmed by the cumulated effect of gastric and small intestinal juice, the *Lactobacillus rhamnosus* IL1 increasing its viability by an average of 20% in the presence of mucin.

Key words: Mucin, pepsin, pancreatin, bile salts, colonyquant, CFU

INTRODUCTION

The development of probiotic use in the diet is a current theme on the functional products market. The viability and stability of probiotics in gastrointestinal conditions, during the transit, the resistance to antibiotics or the presence of other substances with antimicrobial effect have represented a significant challenge for all producers and researchers in the field. To be functional, probiotics must be viable and in sufficient number even after a longer period of time (De Vuyst, 2000). The synthesis of organic acids, antimicrobial peptides and polysaccharides, enhancing the installation of favorable microflora is added to all the above. The novelty in this field is the capacity of such strains to participate to the creation of biofilms, dependant upon the expolysaccharides synthesis in the intestine conditions. (Puangpronpitag *et al.*, 2009; Philip *et al.*, 2009).

The production of probiotics as food supplements requires that the number of viable cells during the manufacture of the product and in the validity period should be of at least 10^5-10^6 UFC g^{-1} (De Vuyst, 2000; Otles and Ozlem, 2003; Yateem *et al.*, 2008). Before the probiotic strains could be used to obtain functional products, they must survive to the processing, to the gastrointestinal stress factors and they should be able to maintain their biological function in the host. All these criteria must be taken into account when choosing the probiotic strain (Chichlowski *et al.*, 2007).

Lately, certain models to experiment *in vitro* the conditions in the human gastrointestinal tract were proposed. They have allowed the study of the lactic bacteria viability and of the influence of the products meant for the balancing of the disturbed intestinal microflora (Pacheco *et al.*, 2010). These simulation systems range from simple ones where the lactic bacteria is treated in solutions of acid medium and solutions of hepatic bile (Favaro-Trindade and Grosso, 2002; Huang and Adams, 2004; Pacheco *et al.*, 2010), to more complex systems that simulate the human gastrointestinal tract to study the probiotic lactic bacteria interactions within the intestinal microbial environment or determine the effect of probiotic lactic bacteria and symbiotic products in the human intestinal microbiota (De Boever *et al.*, 2000; Mainville *et al.*, 2005; Pacheco *et al.*, 2010). An intestinal human tract model that contained four chambers to simulate the stomach, duodenum, jejunum and ileum was proposed by Minekus *et al.* (1995) and Pacheco *et al.* (2010).

Thus, this research will determine the viability in the transit of the *Lactobacillus rhamnosus* IL1 strain through the stomach and the small intestine. The conditions at the

Corresponding Author: Emanuel Vamanu, University of Agronomic Sciences and Veterinary Medicine,
Faculty of Biotechnology, Bd. Mărăşti No. 59, District 1, Bucharest, Romania
Tel: +40742218240 Fax: +40215693492

gastric level were simulated by using pepsin, at various pH values ranging between 1.5-3.

The simulated pancreatic juice contained pancreatin and bile salts, in various concentrations, ranging between 1.5-5. Furthermore, there was tested the influence of casein and mucin on viability, as protectors of probiotic cells. Finally, there was determined the combined effect of the gastric juice and of the simulated small intestine action and there were calculated the mathematical parameters of cell viability and mortality.

MATERIALS AND METHODS

Biological material: The bacterial strain *Lactobacillus rhamnosus* IL1 was maintained in glycerol 20% (Collection of Faculty of Biotechnology, Bucharest), at -82°C. The strain was revitalized by two successive cultures in MRS broth, at 37°C. The experiments were performed in the Industrial Biotechnology Laboratory of the Department of Biotechnology, in the first half of 2010.

The gastric and small intestine juice were prepared according to the method described by Kos *et al.* (2000). In case of simulated gastric juice (pepsin 3 g L^{-1}) there were used various pH values, of 1.5, 2, 2.5 and 3. The simulation of the small intestine juice (pancreatin 1 g L^{-1}) was made at various bile salts concentrations (1.5, 2, 3 and 5 mg mL^{-1}). The mucin and casein influence on the strain viability was determined in the gastric and small intestine juice. A concentration of 1 g L^{-1} in NaCl 0.5% was used and the determination was performed according to the method described by Kos *et al.* (2000). The cumulated effect of the simulated gastric and small intestine juice was determined at a pH of 2 and a bile salts quantity of 3 mg mL^{-1} in the pancreatic juice. All tests were performed in Durham tubes, provided with silicone membrane meant for sampling (Kos *et al.*, 2000; Sarahroodi *et al.*, 2010; Puangpronpitag *et al.*, 2009; Movsesyan *et al.*, 2010).

Furthermore, the effect of trypsin, chymotrypsin and pronase on viability was determined separately for each enzyme. Thus, in a Durham Tube, 1 mL of enzyme solution at a concentration of 1 mg mL^{-1}, 0.3 mL NaCl 0.5% and cell suspension of 0.2 mL were added. In 2 h, the viability was determined in the presence of mucin and casein (Kos *et al.*, 2000; Sarahroodi *et al.*, 2010; Philip *et al.*, 2009).

The viability and the mortality were determined at various pH values according to the method described by Kos *et al.* (2000), in the presence of pepsin and respectively of pancreatin, together with various concentrations of bile salts. The same mathematical indices were calculated as well in the presence of mucin and casein, according to the protection offered to the cell

viability. The critical points were represented by the crossing between the viability and mortality curves (Kos *et al.*, 2000; Sarahroodi *et al.*, 2010; Yateem *et al.*, 2008).

The viability was determined by insemination in double layer, in MRS broth, hourly. The plates were incubated for 48 h at 37°C and the results were read using the Colony Quant and they were registered in the log (CFU mL^{-1}) (Kos *et al.*, 2000; Sarahroodi *et al.*, 2010; Otles and Ozlem, 2003).

RESULTS

In order to be used as probiotic, the tested strain must have good viability in the conditions of the gastric and intestinal transit. The effect of the gastrointestinal transit starting at the level of the stomach is caused by the pepsin, at a pH ranging between 1.5-3. The stationary time at this level doesn't exceed 2 h. Thus, Fig. 1 provides the viability of the IL 1 strain at gastric level. It is noted that the strain viability is directly influenced by the pH. At a pH of 1.5 it represents approximately 97% of the viability obtained for the other pH values, at 0 h of exposure. It lowers to 17.5% within two hours of exposure at a pH value of 1.5. At a pH higher than 2, the strain maintains constant viability after one hour of exposure to the simulated gastric juice. In 2 h, as the pH increases from 1.5 to 2, the viability increases as well and continues to be constant at a pH of 2.5, being of 65.5% as to the initial one. According to the provided data, it results that the strain is resistant to low pH, which is extremely rare in the lactic probiotic bacteria strains.

Mucin is a better protector than casein with respect to the viability of the *Lactobacillus rhamnosus* IL1 strain exposed to the action of the simulated gastric juice. The viability depends upon the pH, but it is higher than when this substance is missing (Fig. 2). The viability values are
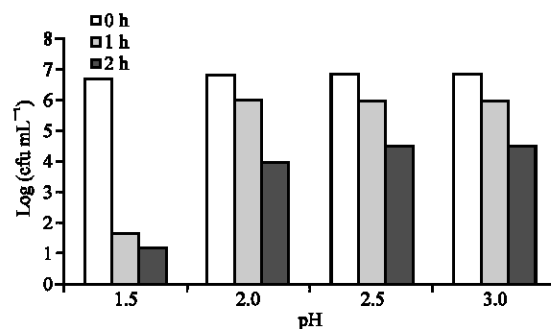

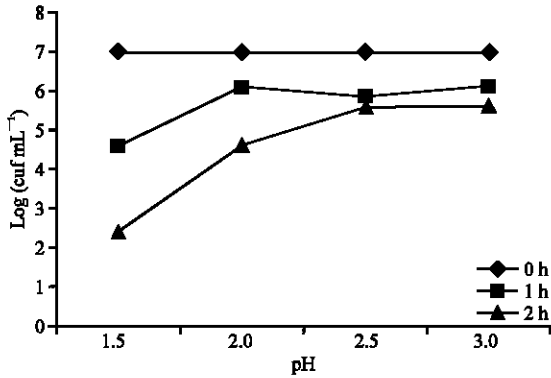Fig. 1: Viability of the *Lactobacillus rhamnosus* IL1 strain at simulated gastric juice exposure

Fig. 2: Casein effect on the viability of the *Lactobacillus rhamnosus* IL1 strain in case of exposure to simulated gastric juice
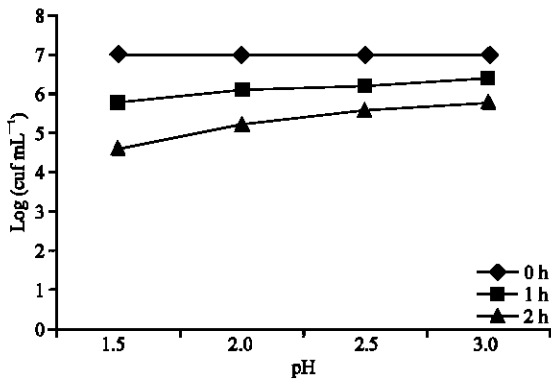

Fig. 3: Mucin effect on the viability of the *Lactobacillus rhamnosus* IL1 strain in case of exposure to simulated gastric juice

higher by 25 up to 50%, at a pH 1.5, both for casein and for mucin (Fig. 3). However, at pH 2, the viability value in the presence of mucin is by 12% higher than in the presence of casein. At a value of 2.5 or 3 of pH, the viability is relatively constant, notwithstanding the presence of casein or mucin. The differences in favor of the presence of mucin, at values of 2.5 and 3 of pH, are of approximately 5%, for an exposure of one or 2 h.

Before testing the viability, in case of exposure to small intestine juice, the influence of other enzymes on the *Lactobacillus rhamnosus* IL1 strain was determined. Thus, a preservation of viability under the action of trypsin, pronase and chymotrypsin resulted, with an average of 6.58 log (CFU mL^{-1}) as to the viability of the strain without enzymes. The value lowers in two hours, due to the action of the three above mentioned enzymes, below 10%.
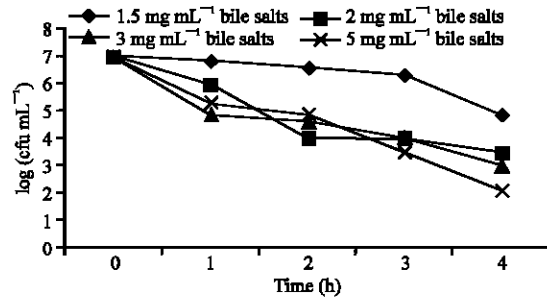

Fig. 4: Viability of the *Lactobacillus rhamnosus* IL1 strain in case of exposure to the simulated small intestine juice
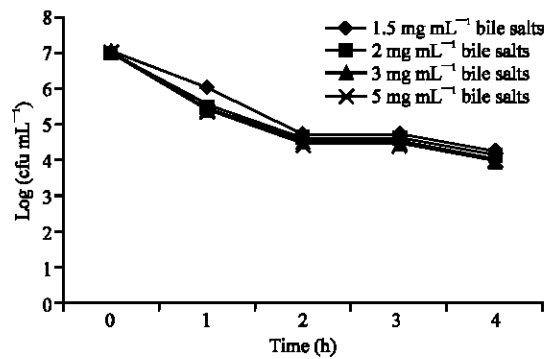

Fig. 5: Casein effect on the viability of the *Lactobacillus rhamnosus* IL1 strain in case of exposure to simulated small intestine juice

In case of direct exposure to the simulated small intestine juice, the presence of bile salts causes the lowering of the viability, mainly due to the increase in the concentration thereof (Fig. 4). An increase of the bile salts of 3 or 5 mg mL^{-1} determines in two hours a significant decrease of the viability, of 25% for 3 mg mL^{-1} of bile salts and 40% for the increase of the bile salts concentration to 5 mg mL^{-1}. It must be noticed that for 2 mg mL^{-1} of bile salts, the viability decreases below 10^5 CFU mL^{-1} merely after an exposure of 3 h. According to this figure, it is obvious that, with the increase of the stationary time in the presence of bile salts, the viability is directly influenced in a negative manner. By doubling the concentration of bile salts, the viability decreases by 40% after an exposure of 4 h.

In case of small intestine juice, the influence of casein and mucin was determined. The two substances, but mainly mucin (Fig. 6), have a protective effect on the viability of the probiotic strain, as opposed with the pancreatin and bile salts effect. Although, the difference is small, the presence of casein (Fig. 5) determines a higher viability decrease. The decrease is directly
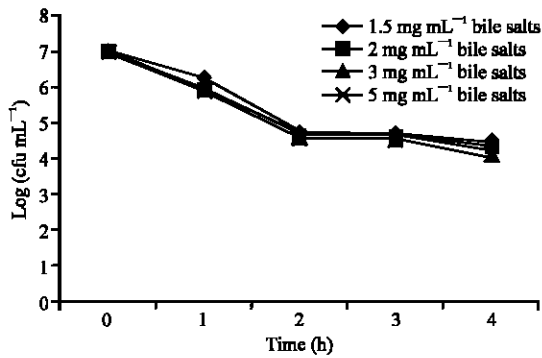
Fig. 6: Mucin effect on the viability of the *Lactobacillus rhamnosus* IL1 strain in case of exposure to simulated small intestine juice
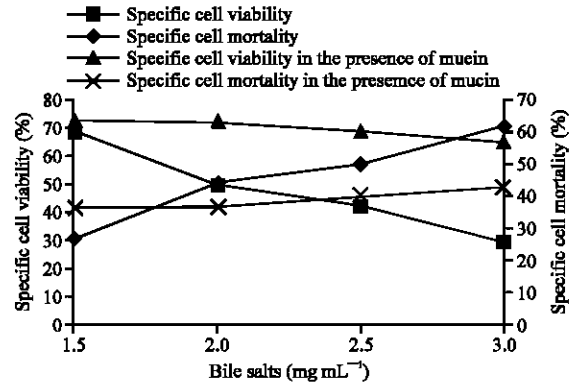

Fig. 7: Specific cell mortality and viability of the *Lactobacillus rhamnosus* IL1 strain in case of exposure to simulated gastric juice

Fig. 8: Specific cell mortality and viability of the *Lactobacillus rhamnosus* IL1 strain in case of exposure to simulated small intestine juice
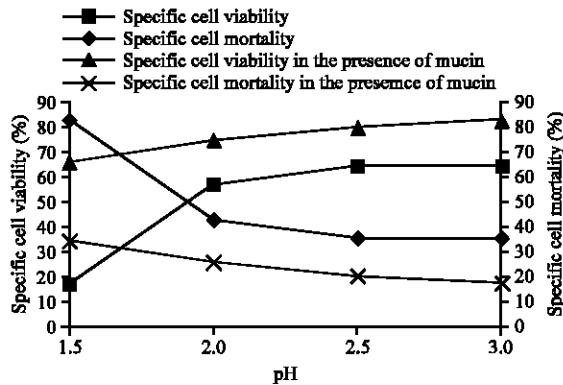
viability at pH of 1.8, according to the literature data, of at least 10^5 CFU mL^{-1} for probiotics (Kos *et al.*, 2000; De Vuyst, 2000).

The same trend is noticeable in case of simulated small intestine juice (Fig. 8). In this situation the presence of mucin protects very well the cell viability, which is supported by the non-crossing of the viability and mortality curves. In the absence of mucin, the strain is strongly inhibited by the increase beyond 2 mg mL^{-1} of bile salts concentration. Thus, at a bile salts concentration of 3 and 5 mg mL^{-1}, the presence of mucin determines an average viability increase of 40%.

DISCUSSION

The protector effect of mucin is noticeable in case of the cumulated action of gastric and small intestine juice on the viability of the IL1 strain. The viability is directly influenced by mucin, although in case of gastric juice action, it is high, of more than 50%, at pH 2. In this situation, the presence of mucin increases the viability value by more than 10%. If the simulated small intestine juice acts on them as well, at a concentration of 2-3 mg mL^{-1} bile salts, the viability is kept at a percentage of 40%, when mucin is present. These data are supported by the previous researches of Kos *et al.* (2000), Patel *et al.* (2008) and Matijasic and Rogelj (2000). The results also represent added data to the findings of Nasrollah (2009) Homayony *et al.* (2008) and Trachoo *et al.* (2008).

Although, it is a regular presence at the level of the gastric mucosa, it provides good protection for the lactic bacteria strains in case of direct administration. The effect of the mixture of mucin with various lactic bacteria freeze-dried strains merely determines an increase in viability, at the passage through the human

correlated to the increase in the concentration of bile salts and in the stationary time. Within two hours from exposure, notwithstanding the concentration of bile salts, the viability decreases by an average of 35%. In two more hours, the viability decreases by an average of 6%. In the presence of protective agents, the viability doesn't decrease below 10^5 CFU mL^{-1}, notwithstanding the concentration of bile salts or the stationary time.

The mathematical parameters of the viability and mortality were determined at various pH values in the presence of different bile salts concentrations. According to the data provided before, it results that mucin is a better protector than casein. It must be noted that the mortality and viability lines don't cross in the presence of mucin, resulting appropriate protection at pH values lower than 2. According to the mathematical calculations, the viability, at pH 2, increases in its presence by 23.1% (Fig. 7). From the same figure, it results that the *Lactobacillus rhamnosus* IL1 strain has appropriate

gastrointestinal tract. This increase of the cell number at the stress exercised by pH 2 and a concentration of 2-3 mg mL^{-1} bile salts contributes to finding new strains of extremely resistant lactic bacteria. Although regularly a viability of approximately 20% is maintained, after such transit, finding strains and conditions able to double the viability is a significant aspect. The researches of Kos *et al.* (2000), Sumeri *et al.* (2010) and Movsesyan *et al.* (2010) are in support of this result, with no disagreement values.

To conclude, it was demonstrated that the *Lactobacillus rhamnosus* IL1 strain is able to survive to the gastrointestinal transit. The presence of mucin as compared to casein determines a viability increase of approximately 20%. The conditions in which the strain has maximal sensitivity were determined, namely pH below 2 and a bile salts concentration higher than 3 mg mL^{-1}, which is significant in order to be able to use the strain in clinical studies. Knowing the protector and the cumulated gastric and intestinal effect on strain viability renders it more competitive when used to create new probiotic products.

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